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Analysis of Exhaled Volatile Compounds Following Acute Superior Mesenteric Artery Occlusion in a Pilot Rat Study

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Abstract

Background—Prompt diagnosis and treatment of acute mesenteric ischemia (AMI) requires a high index of suspicion for timely management. Poor clinical outcomes and delays in surgical treatment are demonstrated even in modern clinical series. Recognition of exhaled volatile organic compounds (VOC's) specific to AMI may facilitate early detection and diagnosis and improve patient outcomes.

Study Design—Adult Wistar Rats (n=5) were intubated and anesthetized and control tracheostomy breath samples were collected using Tedlar gas sample bags. Intestinal ischemia was induced by placing an occlusive clip across the superior mesenteric artery and breath samples were collected following one hour of intestinal ischemia and following 15 minutes of intestinal reperfusion. Gas chromatography was used to identify and measure levels the VOC's obtained and measured retention indices were compared with known values in the Kovats Retention Index.

Results—Multiple retention indices (n=41)) were noted on gas chromatography representing a variety of VOC's detected. Z,Z,-farnesol (C15H26O), an isoprenoid, was the only compound detected which was undetectable during the control phase (median=0 Cts/sec, range=0) but significantly elevated during the ischemic and reperfusion phases(median=34 Cts/sec, range=25–37) and (median=148 Cts/sec. range=42–246), respectively. Three other isoprenoid compounds: E,E-alpha-farnesene, germacrene-A, and Z,Z-4,6,8-megastigmatriene were also detected in all five animals, but their levels did not differ significantly between control, ischemic and reperfusion phases.

Conclusions—This pilot study demonstrates the feasibility of analyzing exhaled VOC's using a novel rat model for acute mesenteric ischemia. These findings may be useful for the development and identification of similar assays for the rapid diagnosis of acute mesenteric ischemia.

Introduction

Acute mesenteric ischemia (AMI) is associated with significant morbidity and mortality especially when time to diagnosis and surgical revascularization is indicated and prolonged.[1, 2] Nonspecific symptoms and the lack of a single diagnostic modality which is pathognomonic for this syndrome may lead to bowel infarction, delays in diagnosis and poor

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patient outcomes. A high index of suspicion by the initial evaluating provider is usually required for prompt diagnosis and treatment to occur.[3]

The detection of distinct volatile organic compounds (VOC's) in exhaled breath by gas chromatography (GC) has been described for other pathologic disease processes.[4, 5, 6] Recognition of volatile markers specific to intestinal ischemia by breath analysis may provide additional information to expedite the decision for surgical intervention. In this study, gas chromatography was used to measure levels of VOC's in alveolar breath before and after abrupt occlusion of the superior mesenteric artery using a rat model. Our aim was to determine the feasibility of detecting changes in VOC levels during a pilot animal model of acute mesenteric ischemia and reperfusion.

Materials and Methods

All studies and care of laboratory animals were performed in agreement with the guidelines of the University of California as established by US National Institutes of Health.

Adult Wistar rats (n=5) each weighing 350 mg were intubated and sedated using general anesthesia (Nembutal). Prior to laparotomy, control exhaled breath samples were collected using Tedlar gas sample bags (1L) at the end of the tracheotomy tube attached to a one-way valve. Surgical exposure of the superior mesenteric artery was performed via a midline incision. Intestinal ischemic injury was achieved by superior mesenteric artery occlusion (SMAO) with a micro surgical clip placed across the vascular pedicle for one hour in the anesthetized rat. Exhaled tracheostomy samples were collected in similar fashion at one hour of ischemia, and after release of the occlusive clamp following 15 minutes of intestinal reperfusion. Visual evaluation of the small intestine was performed at these same time intervals to confirm the presence of bowel ischemia. (Figure 1A and 1B).

Each sample was run through a gas chromatograph (ZNOSE Model 4500, Newbury Park, Ca, USA), and results were matched to the Kovats retention index database to identify VOC's. The Kovats retention index is a standardized parameter used in gas chromatography to compare and identify these compounds.[7] Measured retention indices were compared with known values in the Kovats Retention Index database.

Results

Multiple retention indices (n=41)) were noted on gas chromatography representing a variety of VOC's detected. The most commonly detected compounds are listed in Table 1. Of the multiple gases identified, trimethyldodecatrienol (Z,Z-farnesol-C15H260, molar wt-222.37 g/mol), an isoprenoid, was the only compound detected in significantly elevated amounts in all five animals following one hour of ischemia (median=34 Cts/sec, range=25–37) and at 15 minutes reperfusion (median=148 Cts/sec, range=42–246) compared with pre-ischemic measurements (median=0 Cts/sec, range=0; $p<0.01$). Figure 2 demonstrates Z,Z-farnesol levels at each time point for all five individual animals.

E,E-alpha-farnesene (C15H24, molar wt 204.35 g/mol), an isoprenoid analogue of Z,Z-farnesol was another compound detected in all five animals. The median detected value during the control phase was 227 Cts/sec (range=189–289). Levels of this compound following 15 minutes of ischemia and following reperfusion were 173 Cts/sec (range=28–179) and 164 Cts/sec (range=29–288), respectively. In contrast to Z,Z,-farnesol, these levels were not statistically significant ($p> 0.05$).

Germacrene A (C15H24, molar wt-204.35 g/mol), another isoprenoid similar in structure to Z,Z-farnesol, was also noted in all five rats but levels did not vary significantly between the

control, ischemia, and reperfusion phases. ($p > 0.05$) Levels of this compound were 189 Cts/sec (0–298) during the control phase, 166 Cts/sec (range=0–279) following one hour of ischemia, and 164 Cts/sec (range=29–288) during the reperfusion phase.

Z,Z-4,6,8-Megastimatriene ($C_{13}H_{20}$, molar wt-176.3 g/mol), another isoprenoid, was also present in all five rats during reperfusion. The median value of this compound during the reperfusion phase was 306 Cts/sec (range=25–422). The median values prior to ischemia and 15 minutes following SMA occlusion, respectively, were 227 Cts/sec (range=0–346) and 138 Cts/sec (range=0–414). These levels were also not significantly different.($p > 0.05$)

Discussion

Poor clinical outcomes in patients with AMI and delays in surgical treatment are demonstrated even in modern clinical series'. [1,2] Delayed diagnosis and treatment may occur when radiologic findings are equivocal, or misinterpreted since there are no specific, pathognomonic findings on computed tomography or magnetic resonance imaging specific to the early stages of AMI. Furthermore, an increase in the severity of systemic manifestations and increased patient mortality has been associated with longer times to revascularization.[8] In a study by Kougias, et al, the mean duration between the onset of patient symptoms and surgical intervention was 16 hours. [1] The overall 30-day mortality was 26% and intestinal resection was required in 31% of patients due to infarction and/or necrosis at the time of initial exploration.[1] Another similar review by Park, et al reported a 32% 30-day mortality in their series of 58 patients treated for AMI.[2] Five percent of patients presented with extensive bowel necrosis and unsalvageable disease and 57% of patients required intestinal resection at the time of their initial operation.

To our knowledge, this is the first model to detect exhaled VOC's during and following acute mesenteric ischemia. Our findings from this pilot study demonstrate that elevated levels of Z,Z-farnesol were measured following acute mesenteric ischemia using a rat model. Three other isoprenoids were detected in all five rats, however no significant difference was seen during control and experimental measurements.

Breath analysis can be used in the clinical diagnosis of other pathologic conditions include the detection of acetone in ketotic patients, ammonia in patients with uremia, and a bitter almond aroma suggesting cyanide poisoning[9]. More formal laboratory breath testing has been developed to aid in the diagnosis of other gastrointestinal disorders including: peptic ulcer disease[10], delayed gastric emptying[11], irritable bowel syndrome[12], and lactose intolerance[13].

Although the mechanism and significance of this increased release in exhaled VOC's is unclear in the setting of AMI, decreased intestinal mucosal permeability following ischemia-reperfusion has been well described.[14,15,16] Studies have demonstrated that the initial pathologic alterations of inflammatory or ischemic bowel mucosa occur in the digestive fermentation process, resulting in a change in the type and concentration of gases that are produced by a normal healthy bowel.[17] Systemic absorption of these gases may, in theory, be detected using breath analysis although no prior studies have demonstrated increased levels of these specific compounds during and following ischemic bowel injury.

The only compounds detected in all five animals, Z,Z-farnesol, EE,-alpha-farnesene, Germacrene A and 4,6,8-Megastimatriene, are known to be part of a class of compounds known as isoprenoids, also referred to as terpenoids. Z,Z-farnesol, was the only compound undetectable during the control phase which was significantly elevated during the ischemia and reperfusion phases. This compound originates as a byproduct of the mevalonate pathway via the conversion of the intermediate farnesol pyrophosphate (FPP) which is

known to be a key pathway in the formation of cholesterol, along with key intermediates essential for cellular processes. In addition, farnesol has been implicated in the reduction of serum triglyceride levels in rats.[18]

The oxidation of Z,Z-farnesol into its aldehyde intermediate in the rat has been found to occur mainly[19] in the liver, colon, stomach, and lung and mediated by alcohol dehydrogenases. Although its precise role in the gastrointestinal tract is presently unclear, recent human studies have identified farnesol metabolism in the liver, kidney, and intestines.[20] Farnesene, a close derivative of Z,Z-farnesol, has been found to play a significant role during oxidative injury of eukaryotic cells.[21,22,23] When the autoxidation of this compound occurs in-vivo, cell injury and death have been observed. The inhibition of farnesene production has been shown to significantly decrease oxidative cell injury. [21,22] This effect, however has not been previously demonstrated in mammalian studies

Limitations of our study include the inability to determine whether the compounds detected originate from the gut lumen or are actual by-products of cell injury during bowel ischemia. Measurement of control samples following laparotomy but prior to ischemia would more accurately delineate whether increased levels of VOC's occurred from intestinal ischemia or surgical trauma. . Additionally, the significance of the detectable levels of other isoprenoids in our study is unclear and it is difficult to draw conclusions from this measurement. Additional studies are required to further delineate the importance of these findings. Although the aim of the study was to identify measurable compounds during the early onset of acute mesenteric ischemia, we hypothesized that elevated levels during reperfusion would support the theory that these compounds were more likely to originate in the rat intestine. It is probable that during clamping of the vascular pedicle, the superior mesenteric vein was occluded. This may explain elevated levels of VOC's originating from the intestine due to the presence of accumulated compounds in the absence of splanchnic venous outflow.

Blood samples were not obtained concurrently from the animals for analysis of these VOC's. Theoretically, if systemic absorption of these compounds is the source of these exhaled gases, serologic detection should also be possible. Serologic sampling of both the peripheral and portal veins may also help confirm the origin of these compounds in this model. The addition of mass spectrometry to gas chromatography increases the accuracy of substance determination and reduces the possibility of error inherent to GC alone. We are currently conducting further analysis of these compounds in a similar model using both modalities simultaneously.

This pilot study demonstrates the feasibility of analyzing exhaled VOC's using a rat model for acute mesenteric ischemia. Four distinct isoprenoids were detected by GC in all animals following ischemic bowel injury, and Z,Z-farnesol was the only compound detected in significantly increased levels during ischemia and after reperfusion compared with control. These findings may be useful for the development and identification of similar assays for the rapid diagnosis of acute mesenteric ischemia and facilitate management of this complex disease process.

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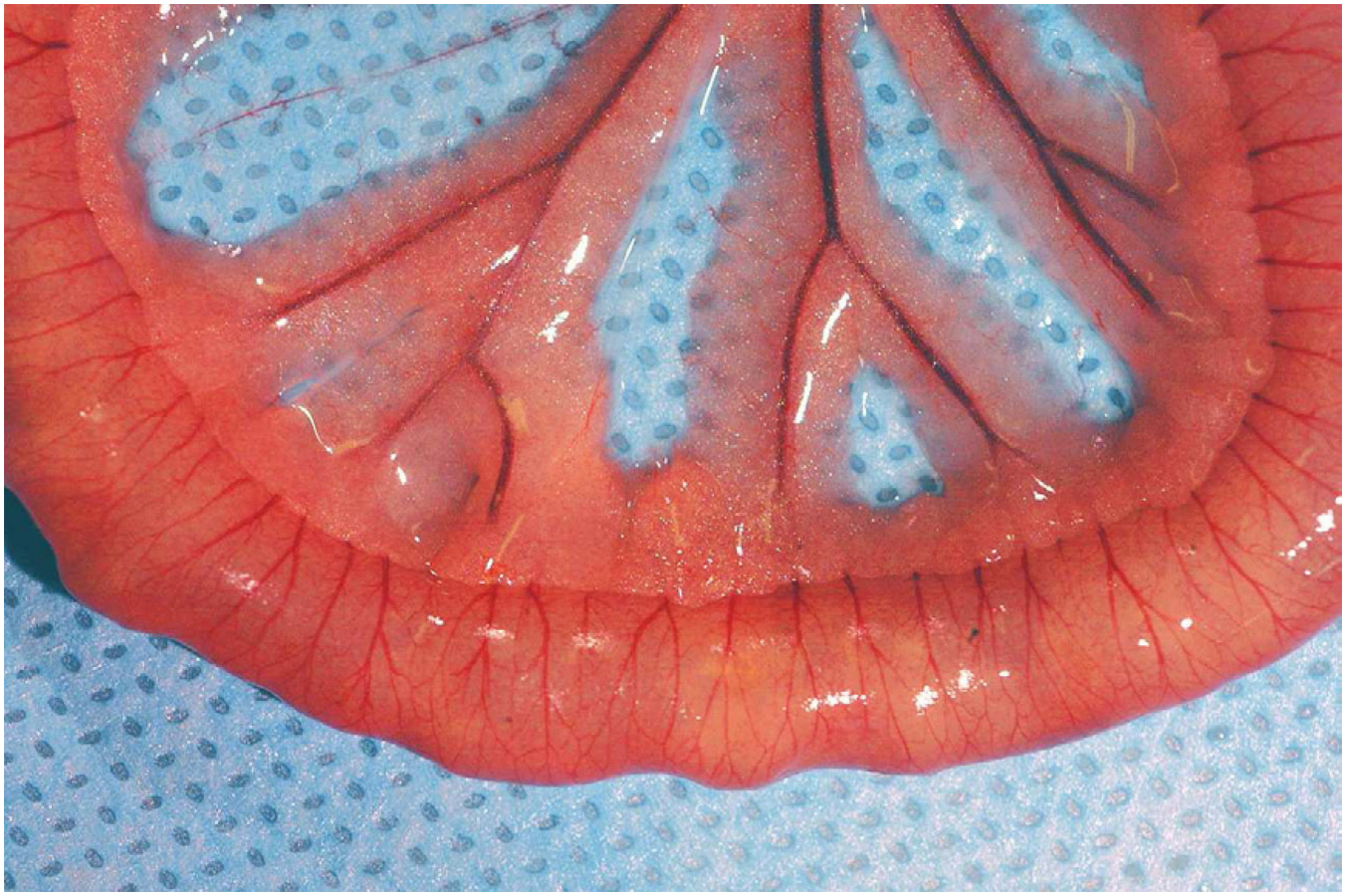

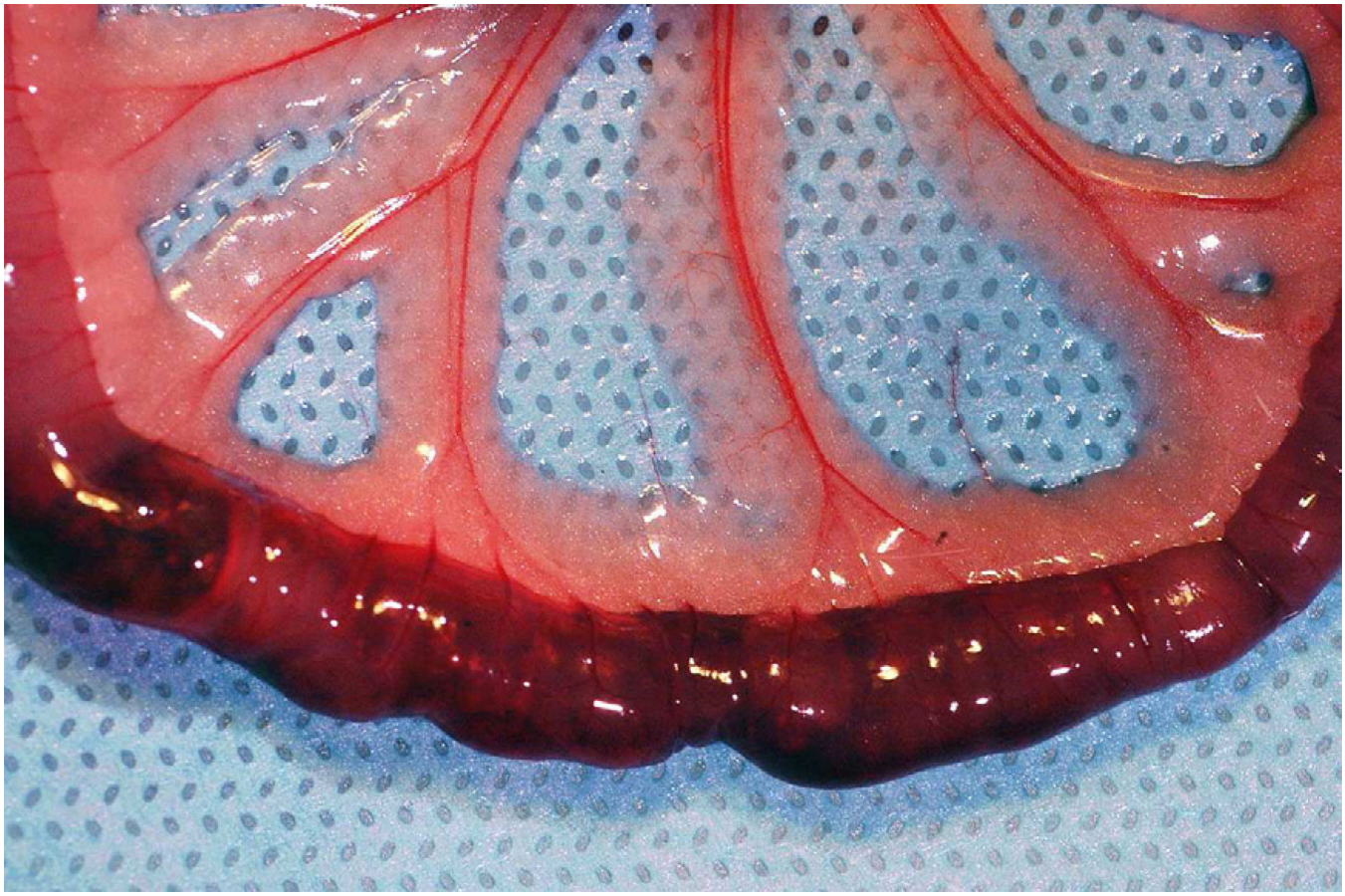

Figure 1.

1A and 1B: Small intestine in the animals prior to (1A) superior mesenteric occlusion and following one hour of ischemia (1B). Visual confirmation of intestinal ischemia was achieved prior to collection of exhaled breath samples

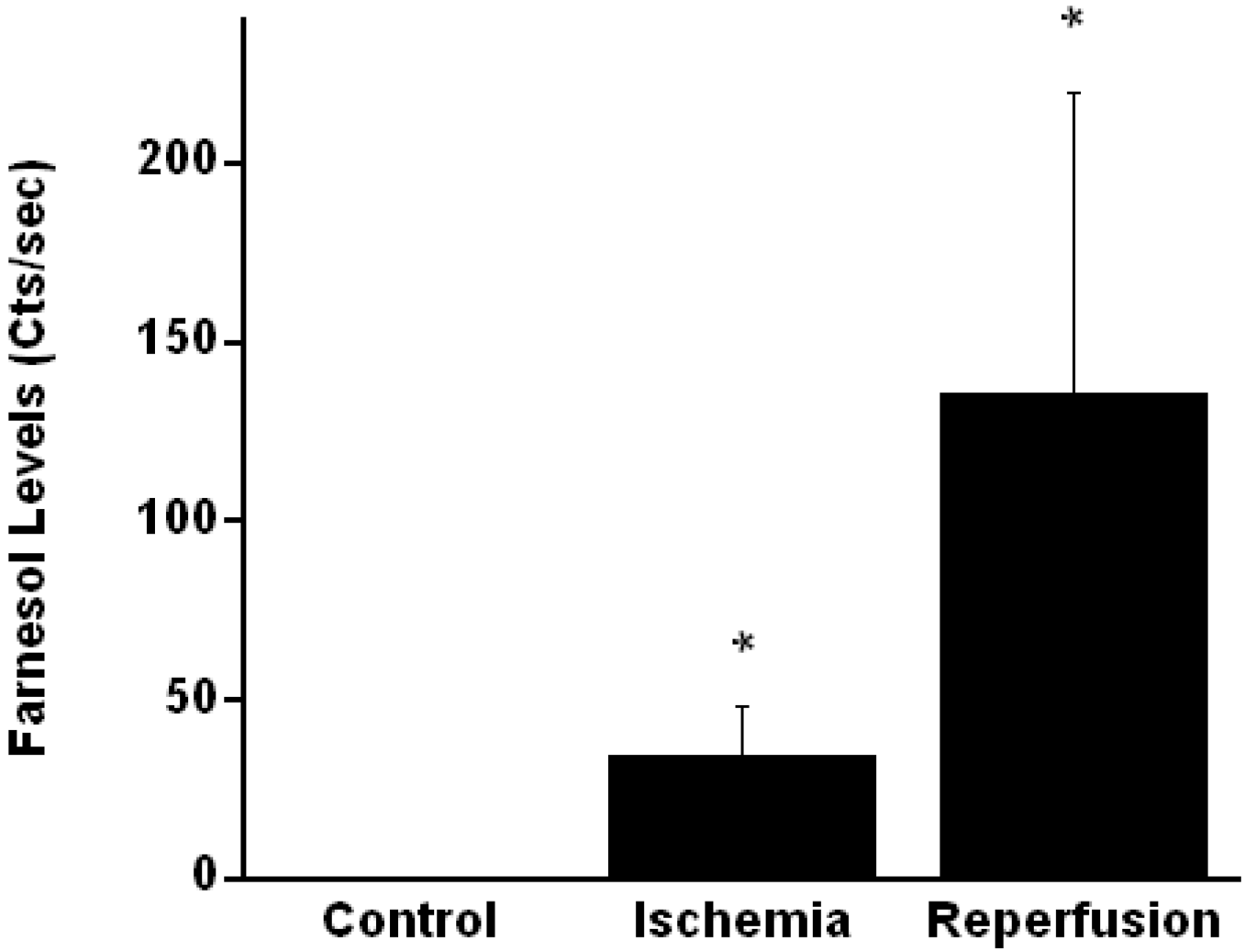

Figure 2.
Figure 2 demonstrates Z,Z-farnesol levels at each time point for all five individual animals.

Table 1

Compounds Detected on Gas Chromatography

			Number of Animals Compound Detected		
			Control	Ischemic	Reperfusion
Compounds Detected	**Common Name**	**Molecular Formula**	**Phase**	**Phase**	**Phase**
(ZZ)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	Z,Z-farnesol	C15H26	0	5	5
(Z,E)-1,5-Dimethyl-8-(prop-1-en-2-yl)-1,5-cyclodecadiene	germacrene A (Z,Z)-4,6,8-	C15H24	3	4	5
4-Methoxybenzylformate	Megastigmatriene	C13H20	3	3	5
(E,E)-3,7,11-Trimethyl-1,3,6,10-dodecatetraene	E,E-alpha-farnesene	C15H24	3	4	5
1-Octen-3-ol	delta-1-octene-3-ol	C8H16O	0	1	2
(Z,E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl acetate	Z,E-farnesyl acetate	C17H28O2	2	2	4
(Z,Z)-4,7-Tridecadien-(2S)-2-yl acetate	2S-Z4Z7-13Ac	C15H26O2	1	0	4
(Z)-3-Dodecen-1-ol	Z3-12OH	C12H24O	2	2	3
(Z)-2,6-Dimethyl-5,7-octadien-4-one	Z-tagetone	C10H160	3	3	3
(Z)-8-Dodecenyl acetate	Z8-12Ac	C14H26O2	1	1	3
Decanal	10Ald	C10H20O	1	3	3
(E)-3,7-Dimethyl-2,6-octadienal	geranial	C10H16O	0	0	2